Agilent's new solution for obtaining routinely quantitative results from NMR measurements.

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The Scope of Analytical Chemistry

Analytical Chemistry is the study of the separation, identification and quantification of the chemical components of natural and artificial materials.

Two fundamental questions:

- What do I have?
- How much do I have?

NMR is amongst the best in answering “What do I have”.

NMR is not that good in answering “How much do I have”.

Methods for Quantification

As with all Analytical Chemistry techniques there are two general categories of methods for obtaining quantitative results.

- Internal standard methods
  - A known quantity of a standard is analyzed together with the sample
- External standard methods
  - A standard sample is analyzed separately and its response is compared to the response of the unknown.

Quantification by internal standard: Requirements for the standard.

- It must be chemically inert (must not bind or react with the compounds of interest).
- Its NMR resonance(s) must be sufficiently far away from those of the molecule or mixture of interest for accurate integration
- It must be available in a pure and inexpensive form
- It must be easily weighed, stable, non-volatile and soluble in the desired solvent
- Its T_1 relaxation time should be similar to the solute of interest
- It should have at least one narrow line that can be accurately integrated
- Not many compound satisfy these and, moreover, people do not like “contaminating” their samples!

Quantification by External Electronic Standard: The NMR approach

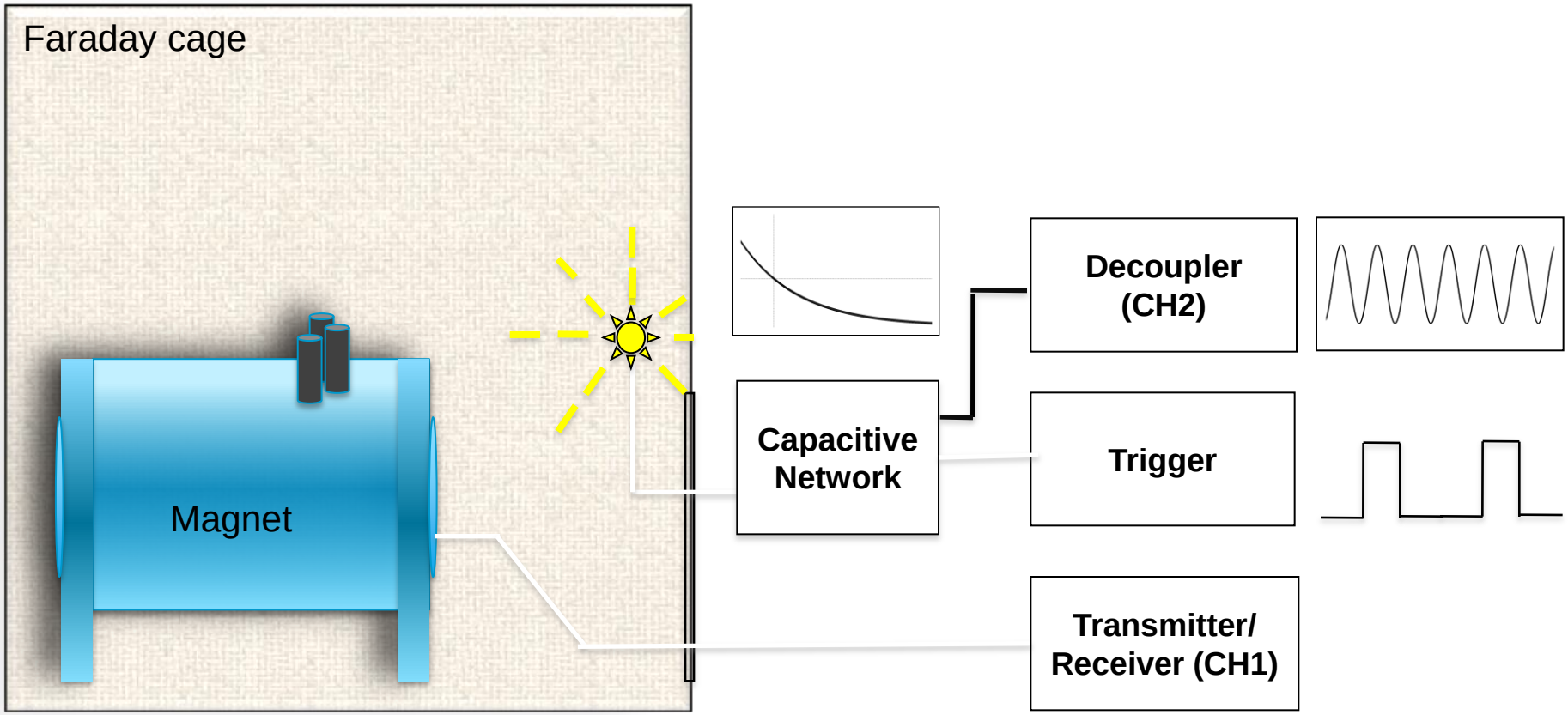
An external quantification technique similar to that used in other analytical methods should be possible for NMR.

One needs to prepare standards in several concentrations and make a calibration curve.

This is rather tedious especially if we consider that NMR has got a dynamic range of around 10^6 with modern hardware, far higher than any other method.

There had been lately a series of methods with external *electronic* reference.

ERETIC "Electronic Referencing To access *In-vivo* Concentrations"



- *Patent: Barantin, L., S. Akoka, and A. LePape, Dispositif d'Analyse Quantitative par Résonance Magnétique Nucléaire, CNRS, Editor. 1995: France.*
- *Barantin, L., S. Akoka, and A. LePape. ISMRM 4th Anual Meeting. 1996. New York.*
- *Barantin, L., A.L. Pape, and S. Akoka,. Mag. Res. Med., 1997. **38(2)**: p.179-182.*

Pros/Cons of ERETIC

Pro

+ Reference signal amplitude automatically compensates for variations in the receive channel
+ Relatively simple setup when using a multi-channel probe
+ Acceptable accuracy for samples with limited variation

Con

− Coupling mechanism for reference signal is more complex than originally implied
− Typically unmatched reference channel, reflection of reference signal is uncontrolled on many probes
− Reference signal amplitude is modulated by different factors other than the observe signal
− Quantitative accuracy can be poor for samples covering a wide range of dielectric properties
− Limits heteronuclear experiments on double resonance probes
− Not easy

Techniques Related to ERETIC

- Add a coil to your probe that is inductively coupled to the observe coil[1]
- PIG: Pulse Into the Gradient. Use the PFG coil as the reference signal generator[2]
- Avoid the probe all together: ARTSI & QUANTUS or no ER[3,4,5]

① Marro, K.I., et al., *Synthetic signal injection using inductive coupling. J Magn Reson, 2008.* ***194(1)****: p. 67-75.*
② Ziarelli, F., et al., *General implementation of the ERETIC method for pulsed field gradient probe heads. J Magn Reson, 2008.* ***194(2)****: p. 307-12.*
③ Mehr, K., et al., *Electronic referencing techniques for quantitative NMR: pitfalls and how to avoid them using amplitude-corrected referencing through signal injection. Anal Chem, 2008.* ***80(21)****: p. 8320-3.*
④ Upton, R. in *ENC. 2008. Asilomar, California USA.*
⑤ Burton, I.W., M.A. Quilliam, and J.A. Walter, *Quantitative 1H NMR with external standards: use in preparation of calibration solutions for algal toxins and other natural products. Anal Chem, 2005.* ***77(10)****: p. 3123-31.*

Do we really need electronic referencing?

NO! With a few calibrations & parameter maintenance in a file (that can be stored with the NMR data) one can make the same adjustments and use an external standard

① Burton, I.W., M.A. Quilliam, and J.A. Walter, *Quantitative 1H NMR with external standards: use in preparation of calibration solutions for algal toxins and other natural products. Anal Chem, 2005.* ***77(10): p. 3123-31.***

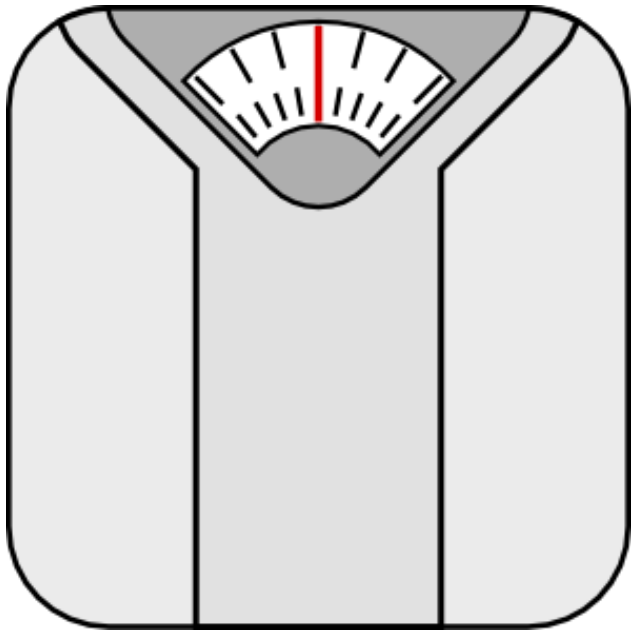
② Wider, G. and L. Dreier, *Measuring protein concentrations by NMR spectroscopy. J Am Chem Soc, 2006.* ***128(8): p. 2571-6.***

A scale remembers calibrations

Your modern NMR is at least equivalent to your bathroom scale ☺

You just need to set the spring!

Your NMR spectrometer is VERY stable over time. If it hadn't then we wouldn't have been able to record 2D or 3D spectra!



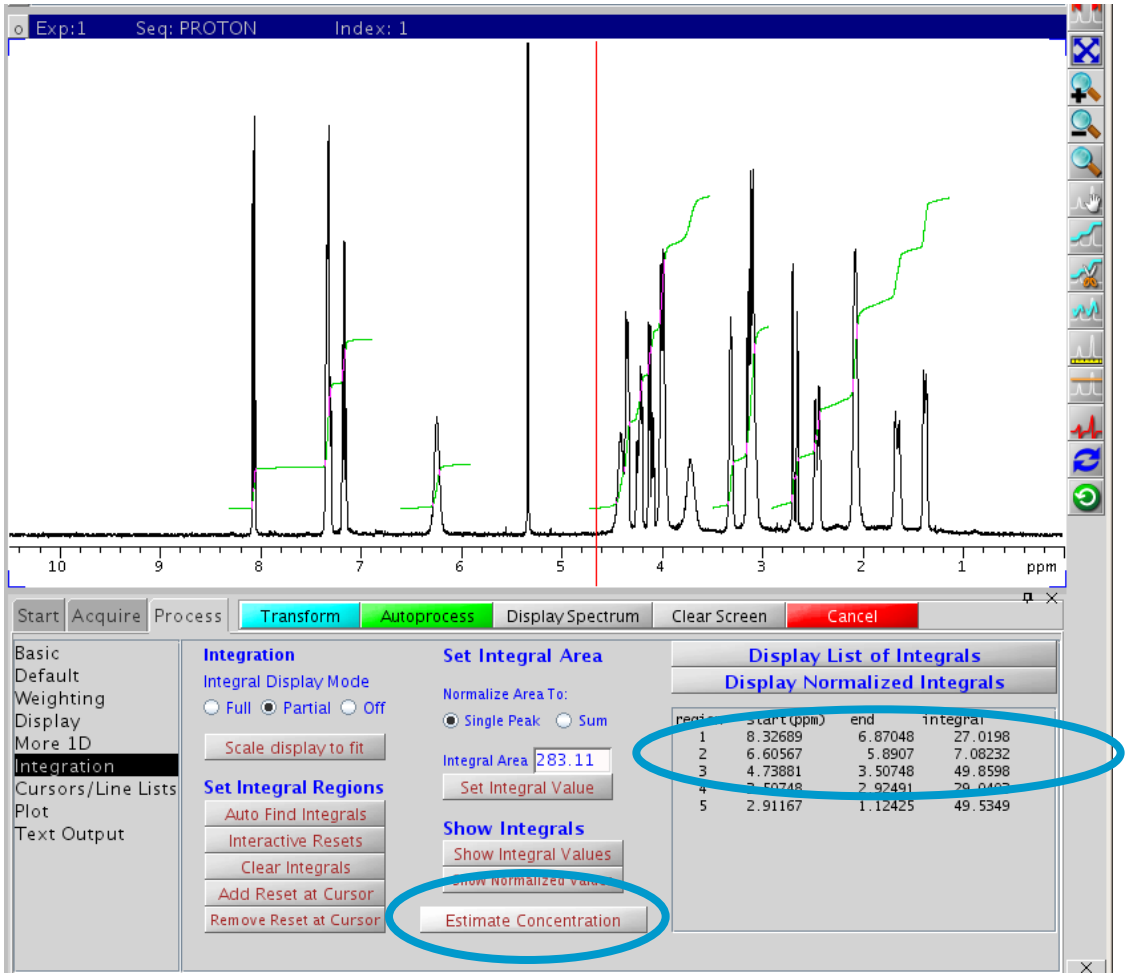
qEstimate – Integrals in Absolute Concentration

- The receiver in Agilent NMR Spectrometers is linear over the entire range so the effect is predictable
- A simple one-time calibration procedure is performed for each NMR probe using a sample of known concentration
- Future samples can be run at any gain or tip angle and once integral regions are defined, **`qEstimate`** displays integral values in actual concentration!
- The relevant entries are stored in the probe file.

H1Qgain	30
H1Qmult	1.00
H1Qins	375.00
H1Qinsref	0.000885203
H1Signal2Noise	650

Set Integrals, click *Estimate Concentration*!

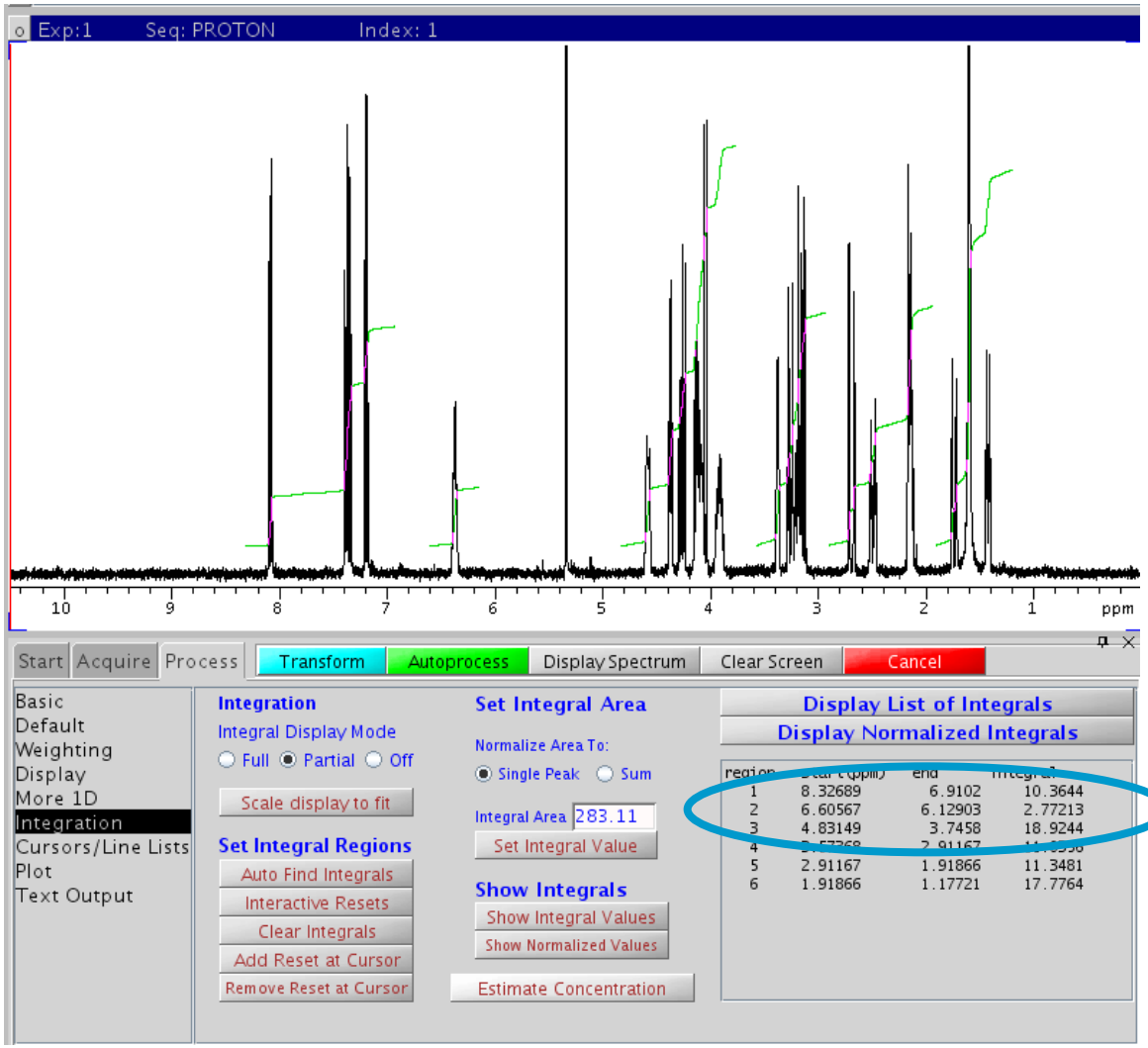
Strychine sample 1:
1.63 mg/0.66 ml ⇒ 7.4 mM
qEstimate: 7.1 mM!



Note that pw90 can optionally be determined automatically for ^{1}H for any sample in VJ 3.0!

Set Integrals, click *Estimate Concentration*!

Strychine sample 2:
0.46 mg/0.53 ml ⇒ 2.6 mM
qEstimate: 2.8 mM!



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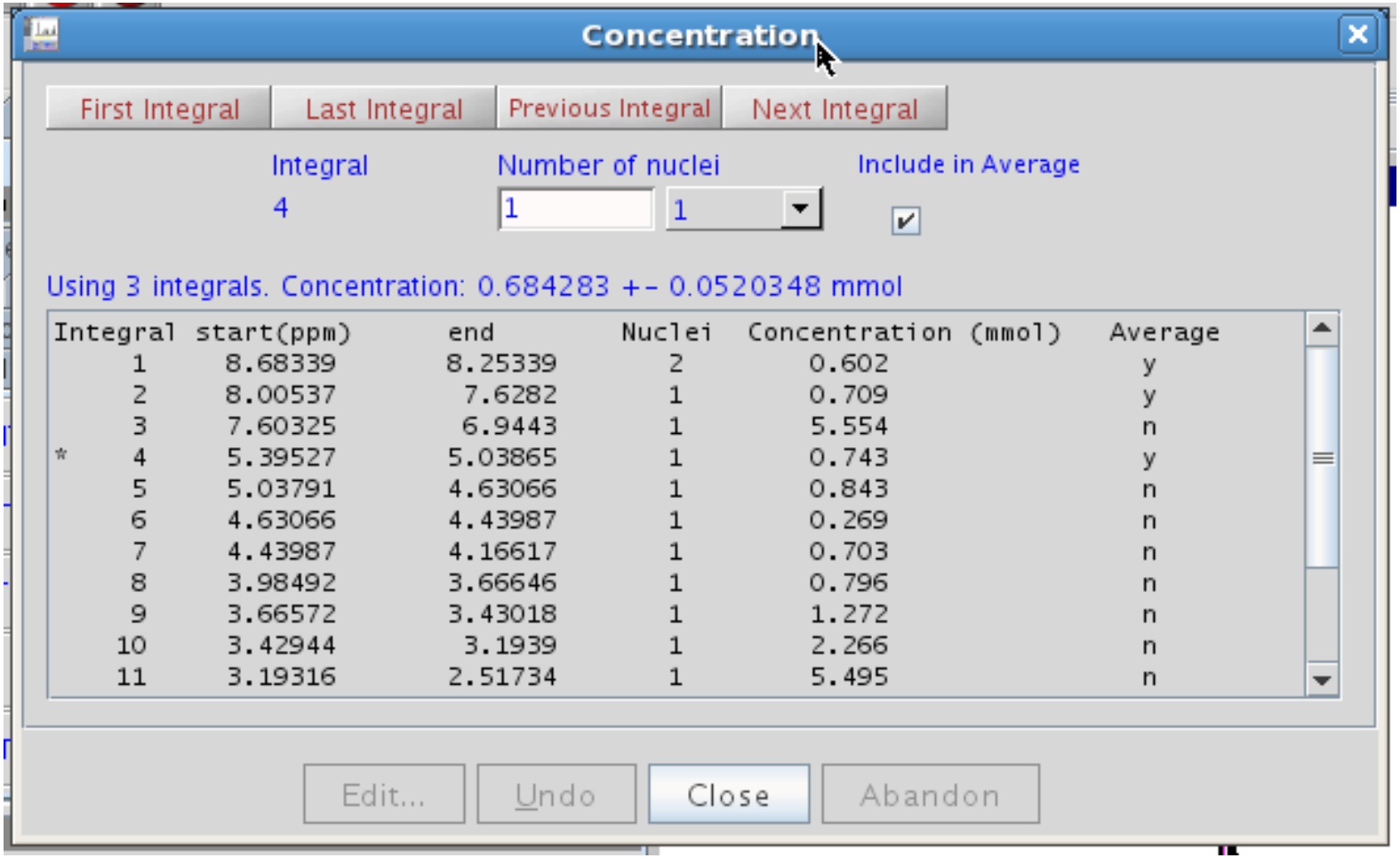
New utility for Easy Integral Evaluations

~400 MW in 130 µl in 3 mm tube. 5 mm OneNMR probe ⇒ 30 µg



Absolute Concentration over 3 orders of Magnitude

Concentration (mM)	qEstimate (mM)	Error %
2000	1948	-2.6 %
1000	1004	+0.4 %
500	492.5	-1.5%
250	250.7	+0.3%
125	121.8	-2.5%
62.5	60.9	-2.5%
31.3	31.0	-1.0%
15.6	16.0	+2.8%
7.8	7.9	+1.2%
3.9	3.95	+1.2%
1.95	2.0	+3.7%
Unknown	70.3	

A New Concept: Adaptive NMR

What if we know the concentration of the sample and we want to optimize experimental parameters?

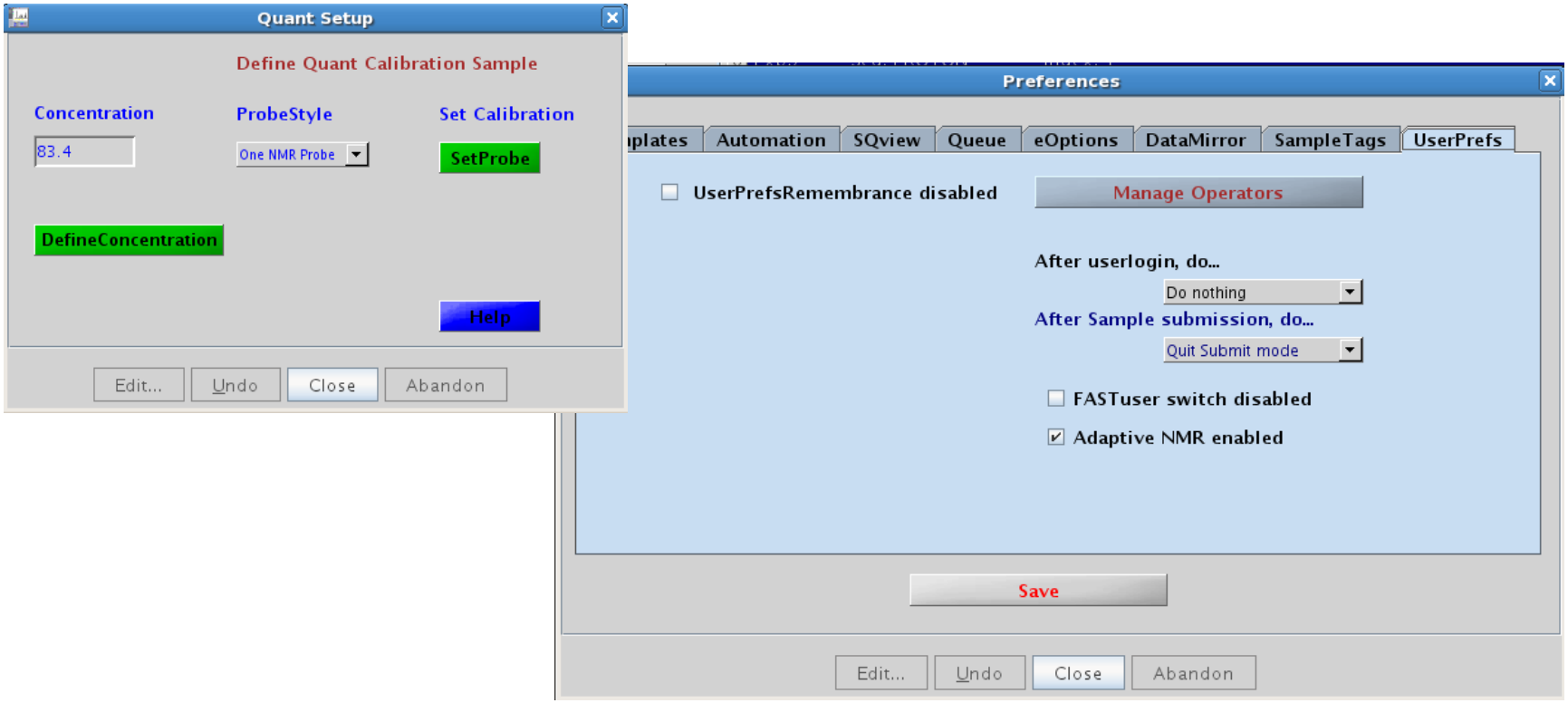
This is opposite to what we did so far.

The software, if required, can estimate the amount of time to be spent on each experiment we wish to record.

The minimum time is defined by the phase cycle (if any) and the desired resolution in F1.

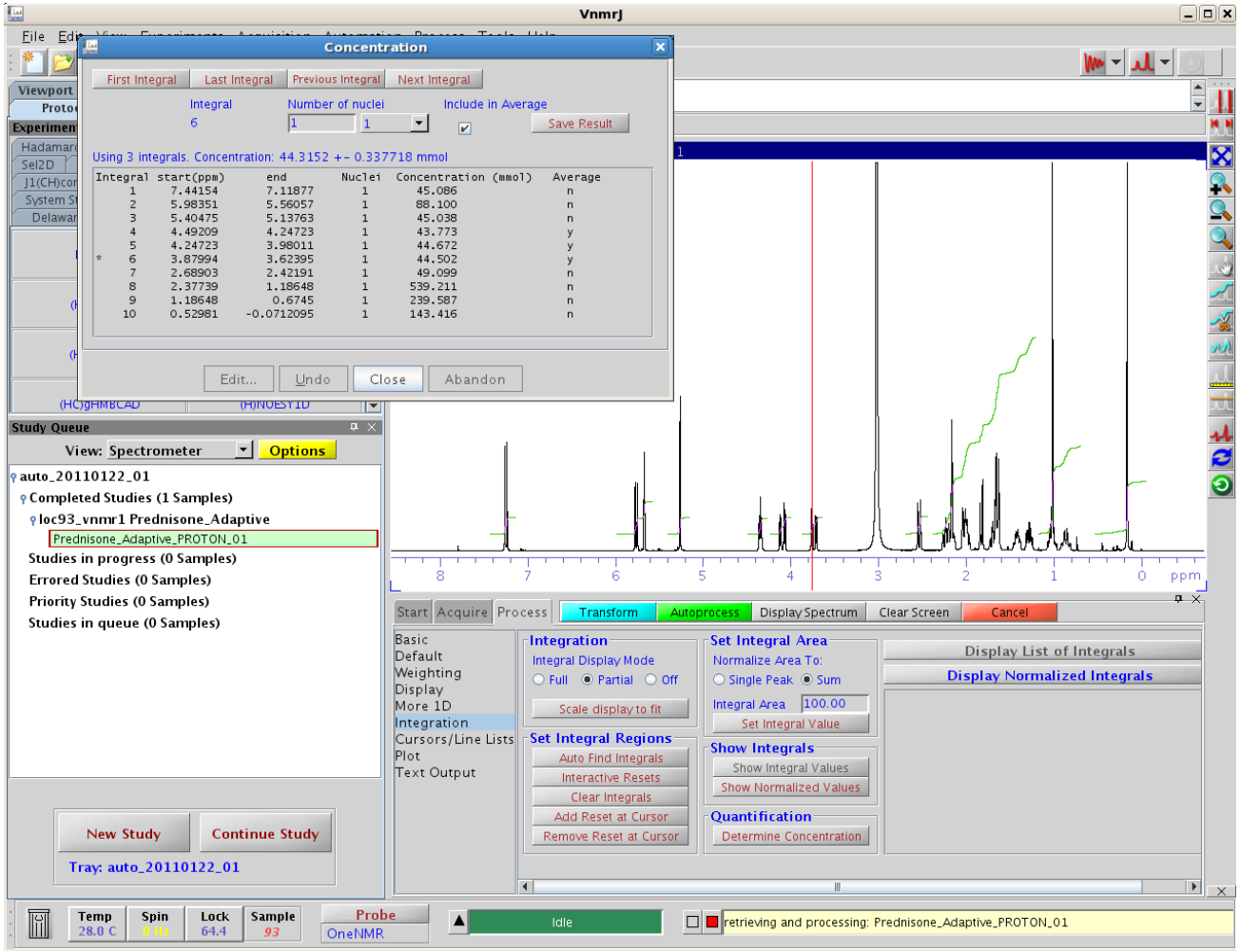
There is no maximum time!

Enabling Adaptive NMR



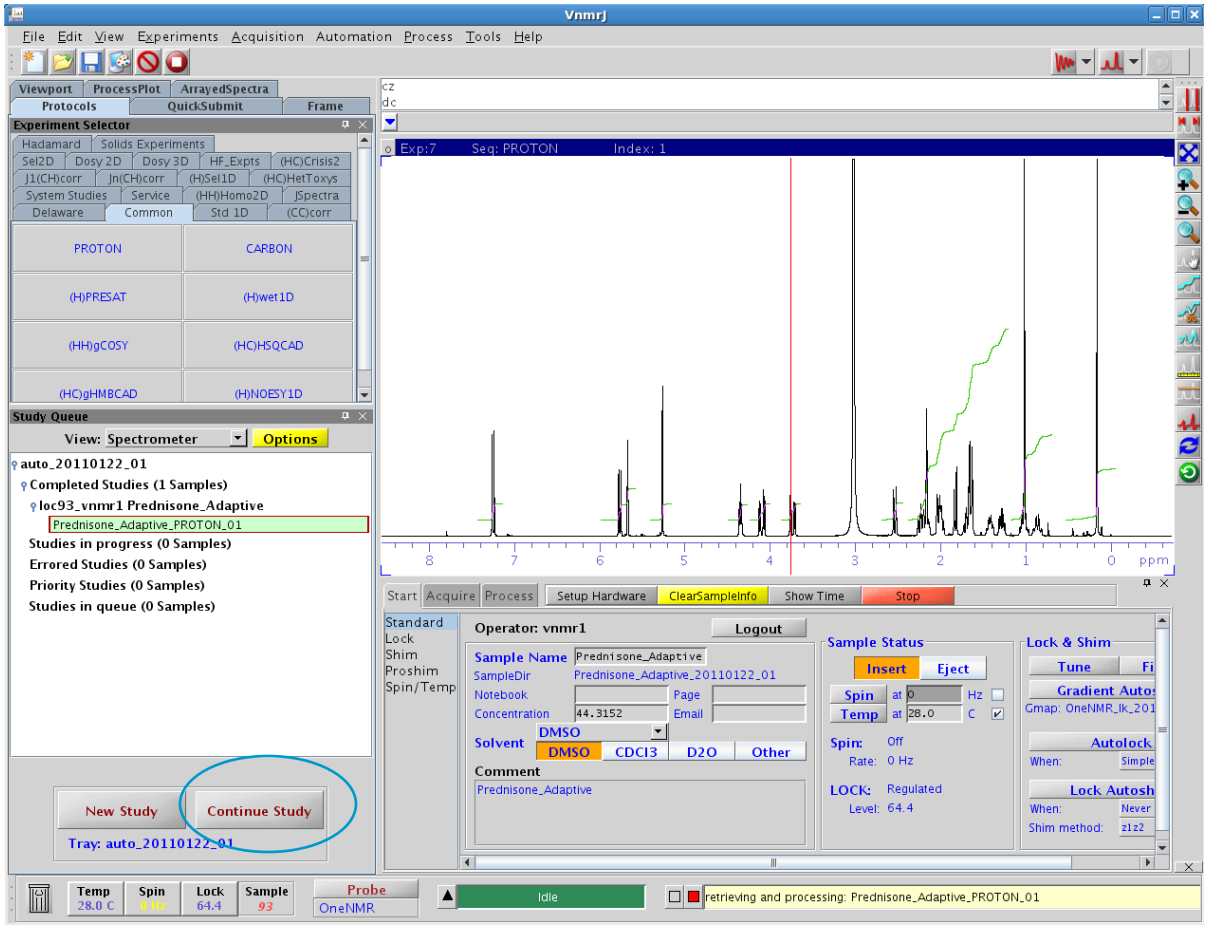
Establishing basic quantification calibrations in easy with any sample of known concentration using the Quant Setup tool. Once those quantification parameters are on the probe file, enabling and using Adaptive NMR is simply checking the box in preferences!

Adaptive NMR in Action



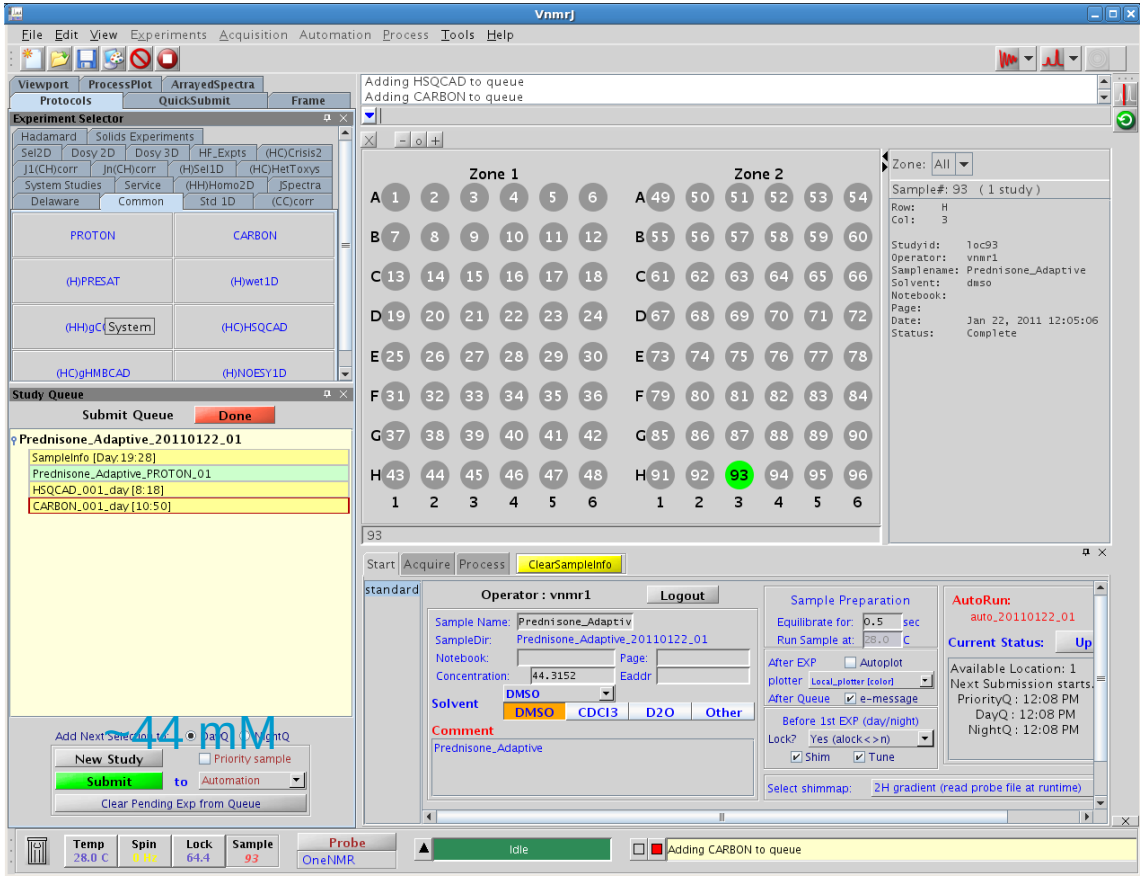
Determine sample concentration and save the result

Continue Study…



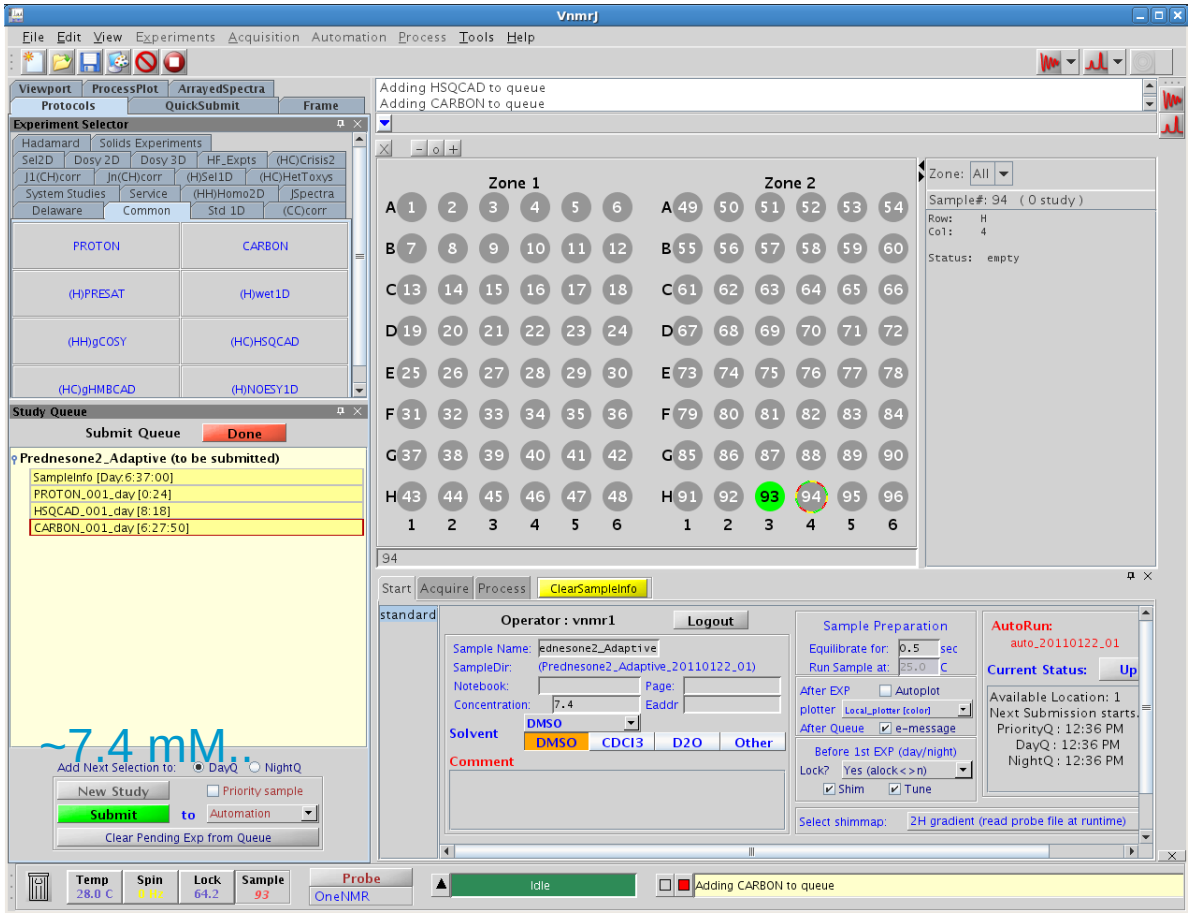
Once the concentration is defined for a sample, the Continue Study function will have access to that value. When a new ewxperiment is requested, the probe file and the Adaptive NMR calibration file (/vnmr/adm/walkupadm/scancalFile) are used to calculated the number of transients required to ensure good experimental results.

Experiments added to the queue automatically set with optimized number of scans



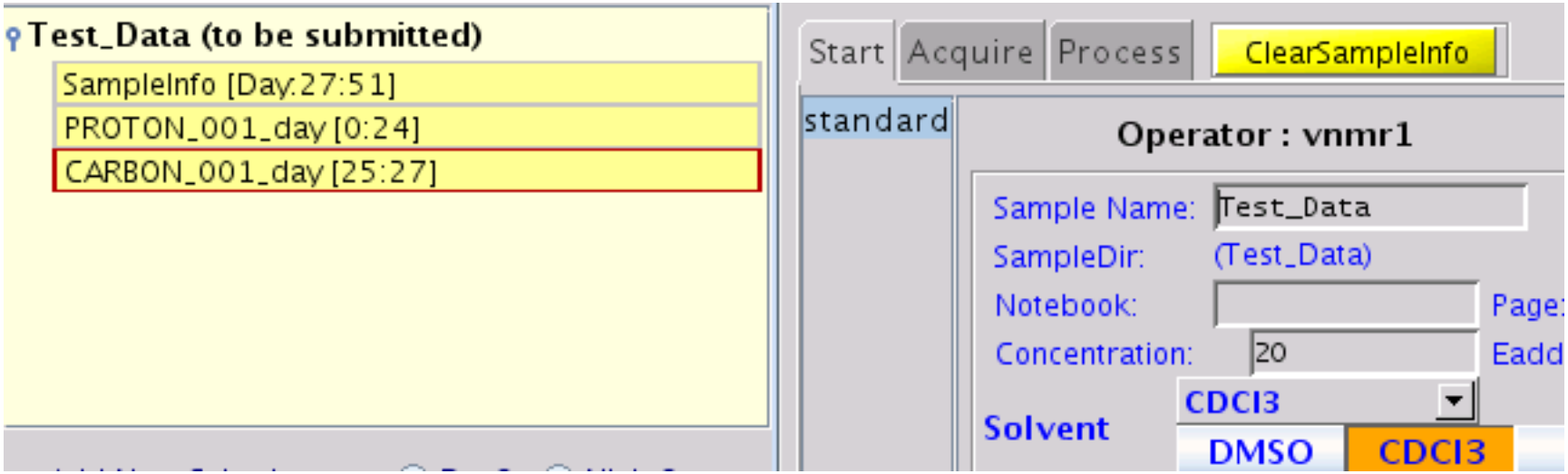
Note the ~44mM concentration and the time for the experiments...

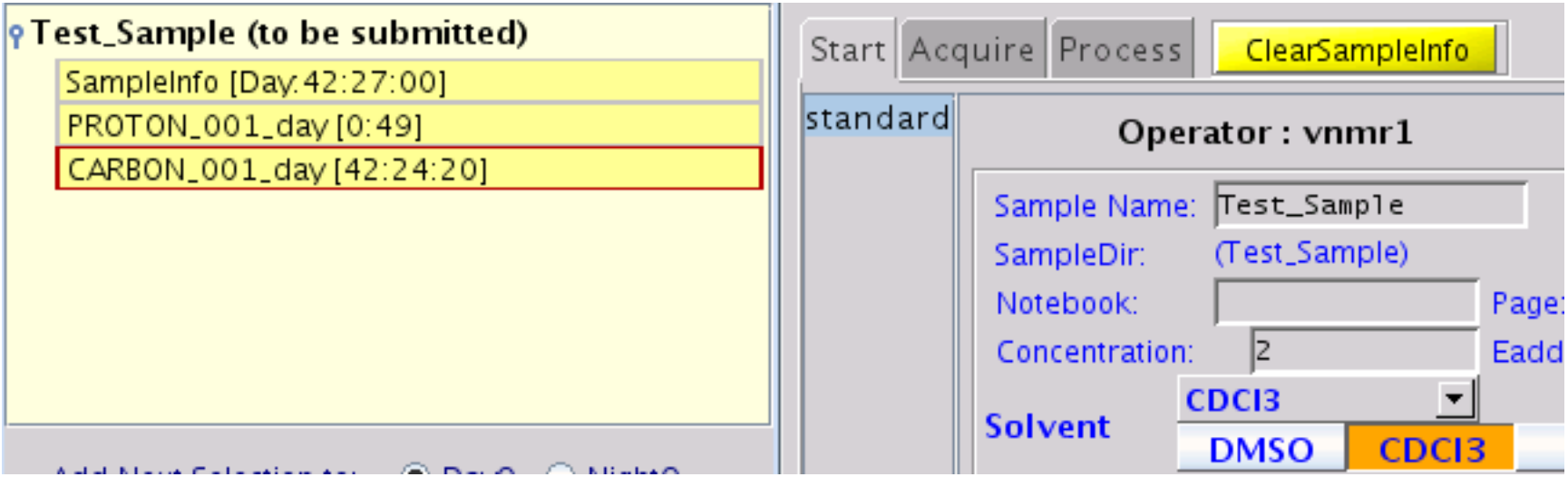
Note how the Carbon experiment is set to run much longer with a more dilute sample!



The previous slide showed setup a ~11 minute CARBON. The concentration of that sample was ~44mM. In the example on this slide the sample concentration was ~7.4mM and a 6.5 hour CARBON was setup. The signal to noise target for any experiment is tuneable!!

Adaptive NMR in action

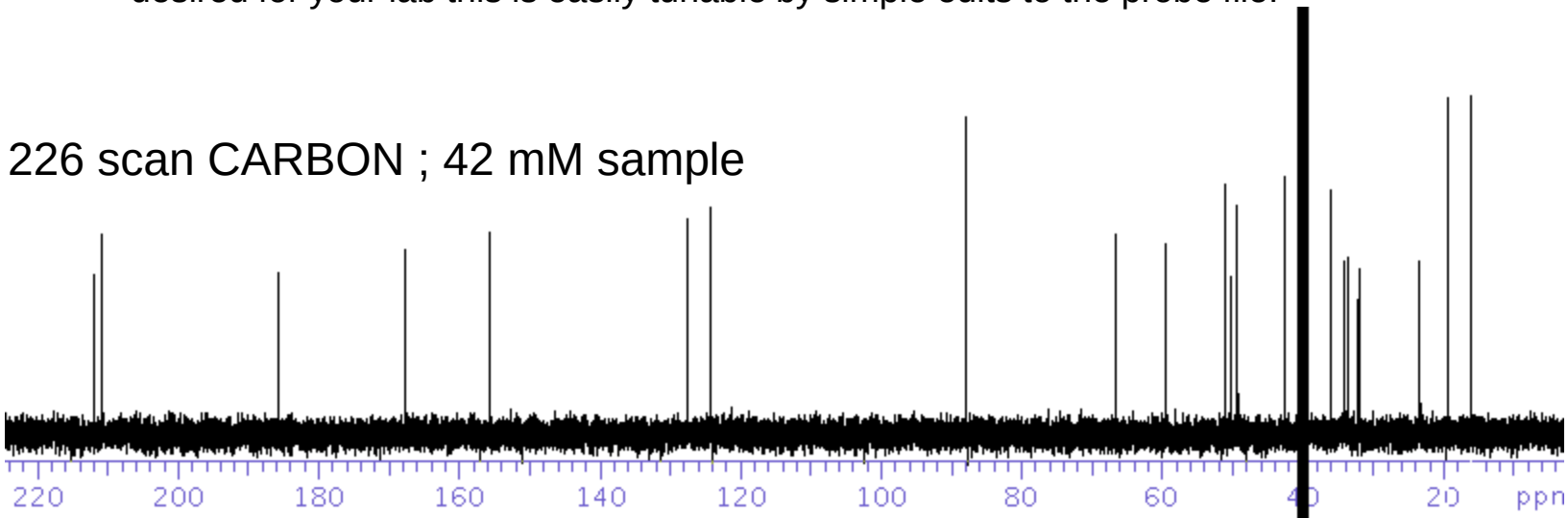




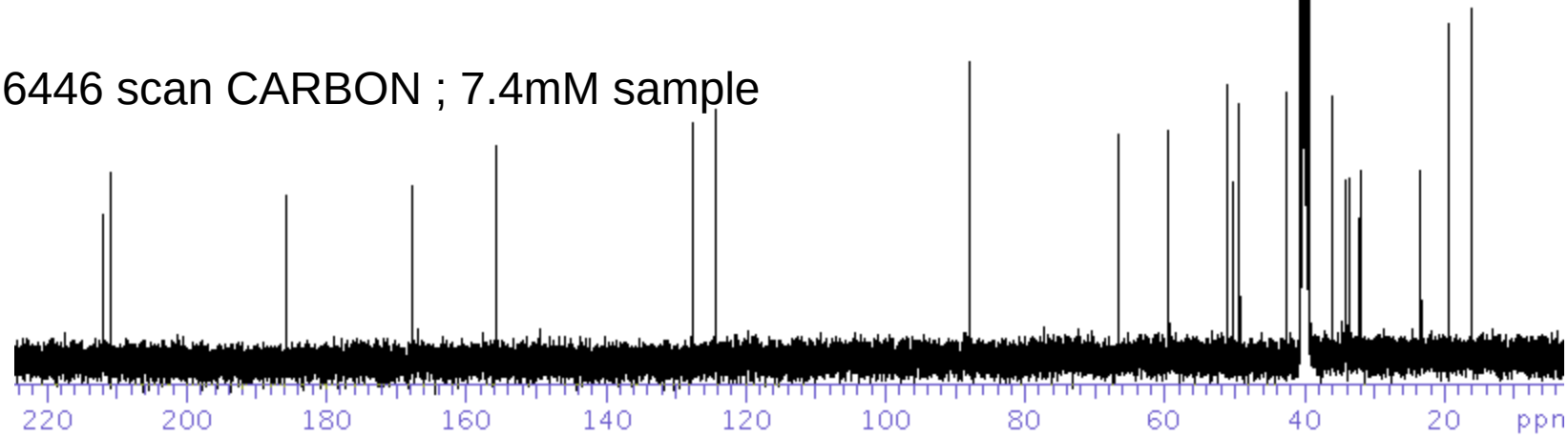
Adaptive NMR in Action

Here are the two CARBON spectra obtained in automation with the automatic setting of # of scans by Adaptive NMR. If more or less signal to noise is desired for your lab this is easily tunable by simple edits to the probe file.

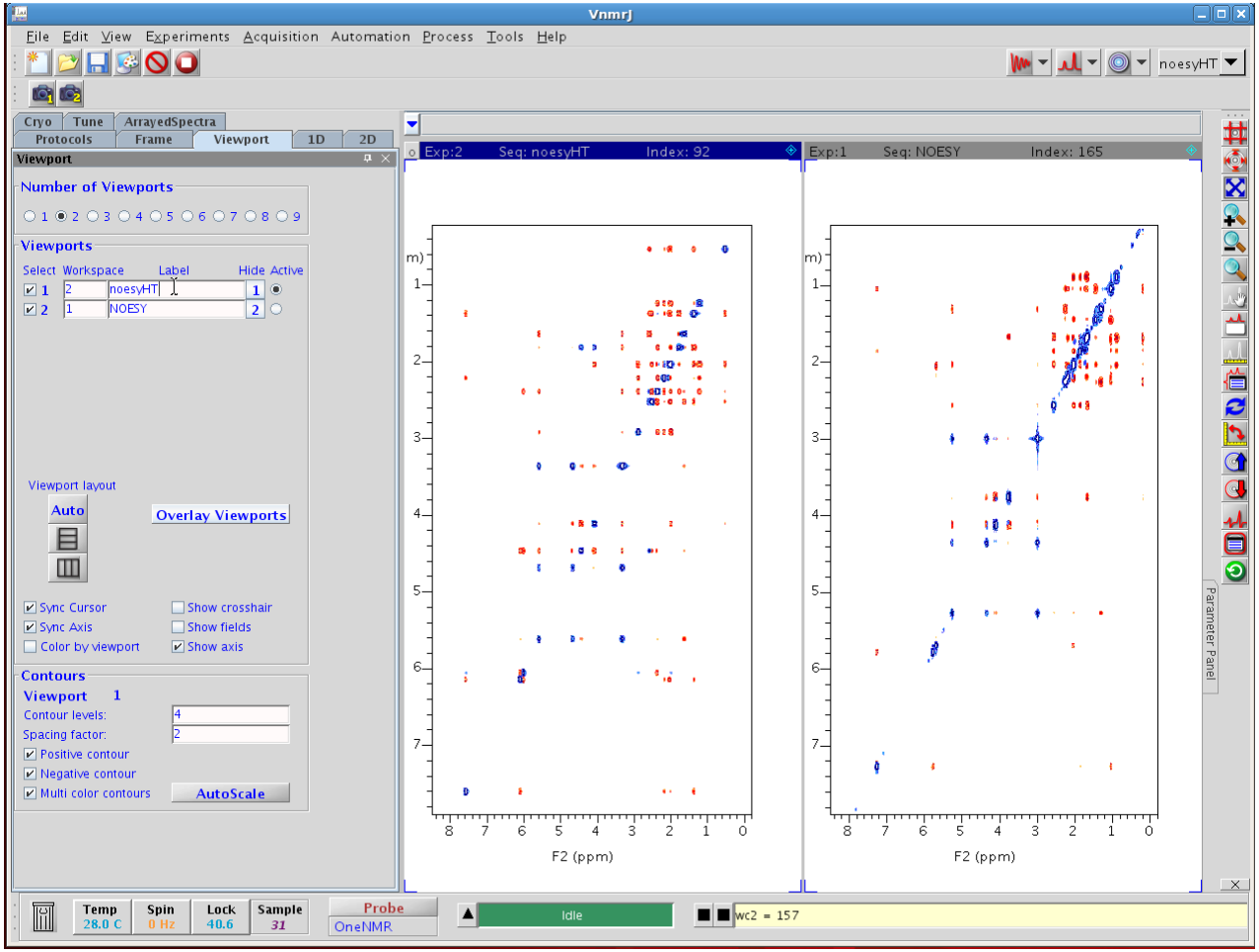
226 scan CARBON ; 42 mM sample



6446 scan CARBON ; 7.4mM sample



40 min NOESY & 4 min noesyHT – both acquired in same automation run, # scans predicted



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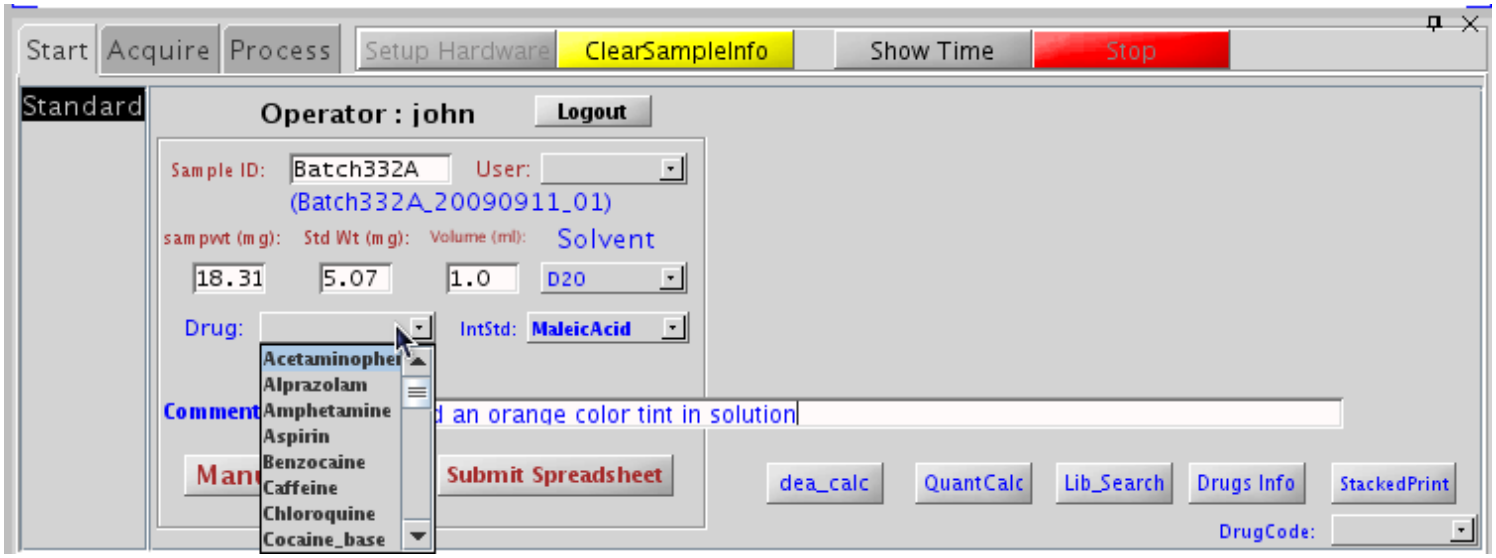
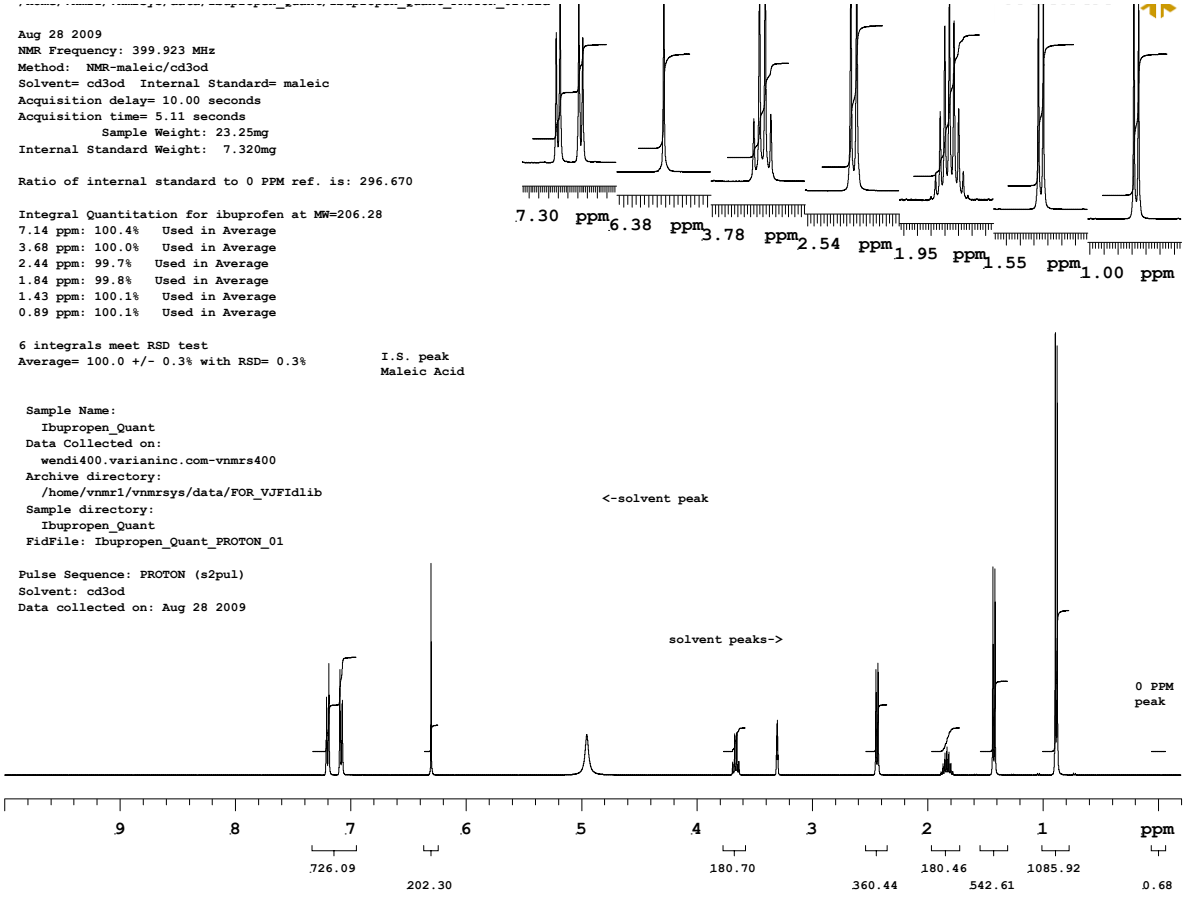
Flexible and Customizable Sample Entry Tools

- If you work with a defined set of substances for Quantitative analysis, use a configurable menu for sample entry
- Batch submissions by spreadsheet



Output *more* than a Simple Plot

Software with the *flexibility* to output reports and not simple plots.
Example PDF ibuprofen report



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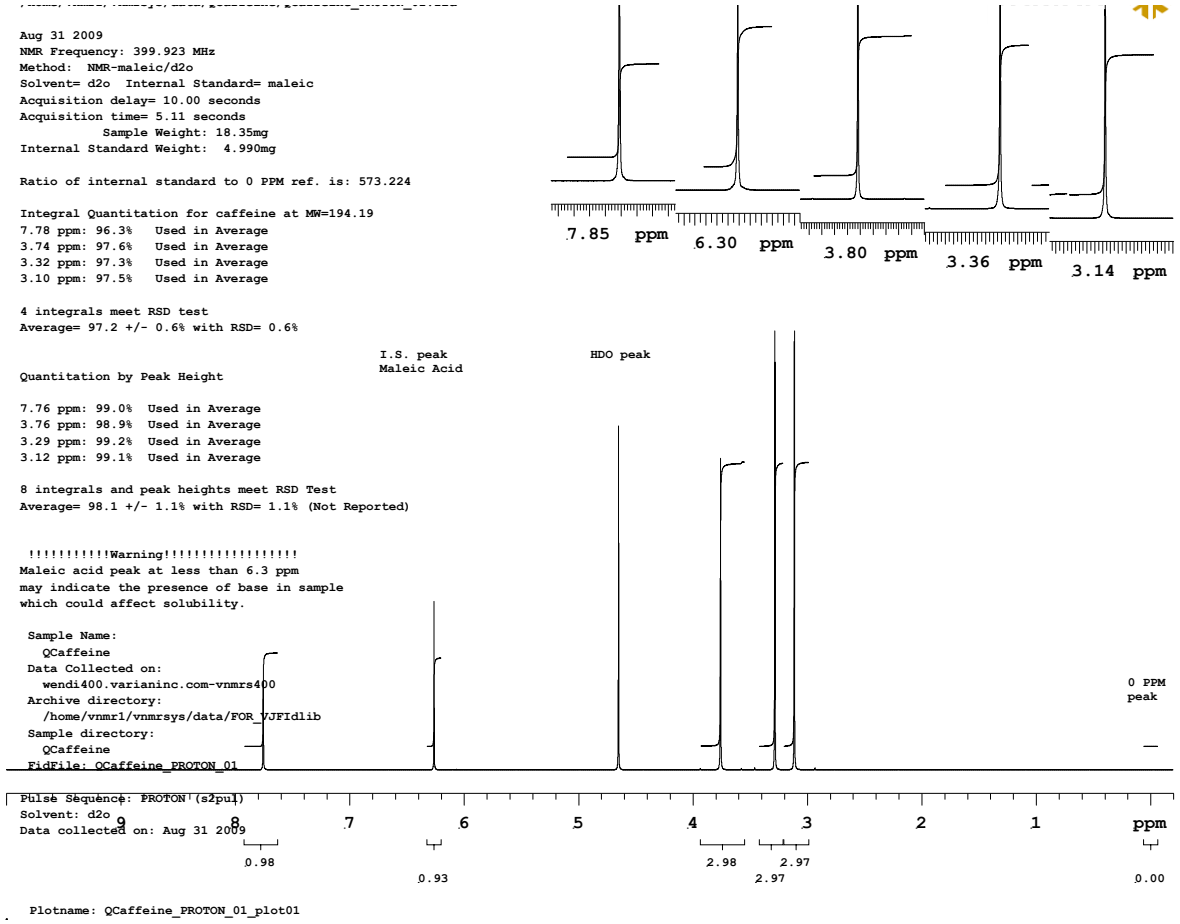
Caffeine QA Report with Q by peak height included

Note: this caffeine sample was labeled “98%”



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Thank you!

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